

# Biomimetic Polyaminoacids as Precursors for Optical-Active Intelligent Materials

G. Popova, Research Laboratory "Molecular Materials"  
Mendeleyev University of Chemical Technology, Moscow, Russia

## Abstract

Three main types of light-sensitive polyaminoacids (PAA) such as: rod-like dyes contained; photoactive rotaxanes; inorganic-organic cyclophosphazene hybrids are considered in context of intelligent materials creations. Light-manipulative biomimetics preparation involves strategy via combinatorial synthesis; computation and modelling of possible molecular architecture; self-organization and supramolecular phase formation; function on nanoscale; stimulus-responsive study; intelligent materials formation.

Modified polyglutamic acid with dyes regular set possesses specific ability to self-assembly with cooperative rearrangement under outer temperature (carbocyanines bonded), pH (fluoresceine bonded). Ion-sensitivity of photoactive peptide (oligo-glutamic or oligo-glycine) rotaxanes is promissive feature for molecular switches construction. Inorganic-organic hybrids with cyclophosphazene cores (star-like design of poly-glutamic or poly-alanine) are the convenient matrices for different fragments immobilization that can result to light-controlled conformations of PAA in variable states: solution, gel, film.

## Introduction

During last decade, a lot of investigators paid attention to Stimulus-responsive-"intelligent"- material creation that could work in nanoscale. The formation and function of these substances in Supramolecular phase is key fundamental problem nowadays [1]. Different building blocks, which are sensitive to outer factors as temperature, pH, illumination, electric and magnetic fields, etc, can be used for material construction. These materials may exist in various forms, such as solutions, gels, liquid crystals, layers, films [1, 2, 3]. Biomimetic "intelligent materials" have a good perspective for imitation of the natural objects. From this point of view, the modified artificial polypeptides cause current research interest. They can form the  $\beta$ -sheet or  $\alpha$ -helical conformations as it is in real proteins. The particular case is polyaminoacids consisting of only one type of aminoacid. They can produce the helical rigid-rod macromolecules deserving a special study. Particularly, introduction of different chromophores - dyes, photochromes, luminophores - results to new properties, closed to natural. The well-defined rod-like and hairy-rod structures of polyglutamic acid derivatives (PGAD), their abilities to conformational changes, reversible Arrangements permit to investigate variable properties useful for biomedicine, molecular electronics, optical and sensor devices [3, 4, 5].

Intensive research were devoted to peptide based rotaxanes [6, 7]. The presence of multicentres for hydrogen, ionic and other bindings is favorable for controlled moving construction. Rotaxanes can be used as sensitive elements for "molecular machines" [8] as models of light-, electro- or ion-driving shuttle or switches. Previously, cyclopeptides containing one or two crown-ethers produce Supramolecular complexes with functional Spiropyranes. Quantum Chemistry methods were applied for Simulation, Modelling and Optimization of possible Molecular Architectures of photoactive complexes. The size of cyclopeptide cavity defines intra- or intermolecular complexation. Several co-polymers were synthesized and tested as sticks in depend upon ring moving. Reversible process irradiation / metalloion containing results to switches available to intelligent systems creation.

Mimic protein tertiary structure is founded on the template-assembled concept and is one of the convenient methods that have been used for this aim. Linear or cyclic peptide templates have been used for covalently binding of parallel  $\alpha$ -helical peptides [9, 10, 11, 12]. Cyclic peptides, tris-bipyridyn metal complex, porphyrine were applied as templates for formation of folded tertiary peptide structures [9, 10, 11]. Tetraphenylporphyrin as a more rigid template for a four-helix proton channel building [12]. The nine-residue peptide template has been utilized for chemoselective ligation [12] of unprotected peptide segments resulting in the total chemical synthesis of protein analogs of native topology. An amido amine dendrimer cyclotriphosphazene [13] have been successfully applied for assembling oligopeptide chains by graft polymerization on surface. It was found that the organized oligopeptide onto a dendrimer template results in drastic enhancement in helicity of the peptide segment.

All these points became reasonable basis for our investigations on peptide Intelligent Systems construction. Presently we discuss several properties of biomimetic polyaminoacid derivatives in solutions, monolayers and Langmuir-Blodgett films. Principal strategy such as combinatorial chemistry - computer modelling and calculation - supramolecular synthesis and supramolecular phase formation - function in nanoscale - stimulus responsive study - intelligent materials creation was developed for each type of substances. Synthetical details and identification are outside of this proceeding.

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## Results and Discussion

### Polyglutamic Acid Modified with Dyes

Linear rigid-rod polyglutamic acid with regular set of dyes was obtained by covalent bonding of light- and electro-sensitive carbocyanines with polyglutamic acid. The reversible colour transition on heating in LB-films of polyamino-acid containing dyes have been studied using absorption, Stark spectra and electroconductivity. Maximum shifts of the absorption spectrum are explained by possible interconversion of dyes and induced changes in macromolecule formation of different kinds of aggregates, conformations and hydrogen bonds. These polymer films are perspective for their thermosensitive properties.

The monodisperse rod-like luminescent  $\gamma$ -derivatives of poly-L- $\alpha$ -glutamic acid, their ability to Self-Organization in Solutions and monolayers as well as their response to outer influence are objectivity of this research as well.

Incorporation of dyes in polymer matrix permits to modificate their common properties, to construct novel high-tech materials for using as sensors, transducers, amplifiers in different molecular devices.

Our efforts have been concentrated on the preparation of polymer derivatives of light- and electro-sensitive cyanine's dyes, according to Figure 1.

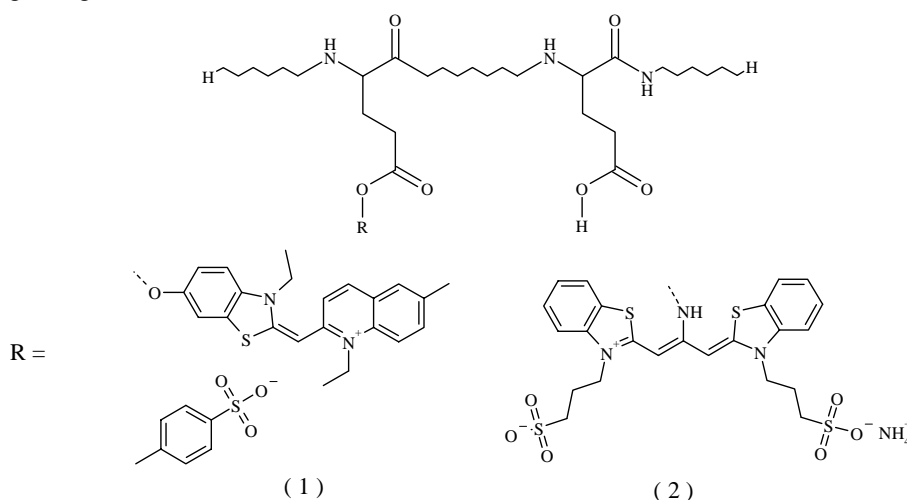


Figure 1. Chemical formulas of carbocyanine dyes and polymer matrix.

Polyglutamic acid (MM 6000) was applied as polymer matrix, containing carbocyanines (36% for compound 1 and 28% for compound 2). When sensitive fragments are attached to polyaminoacids, their reactions stimulate structural changes, i. e. photoelectroinduced conformational changes are accompanied by large and reversible changes in physical properties, what is very suitable for sensor devices [5].

Early we have shown the existing of ordered structures - H-aggregates in LB-films of cyanine dyes covalently bonded with polyglutamic acid [14]. These films detected irreversible change colour under UV-irradiation. Polyglutamic carbocyanines (1, 2) are reoriented or decomposed in concern upon power and time of irradiation. The thermochromic reversible effect was detected for LB-films of polyglutamylcarbocyanines as well. Similar effect of reversible colour change under heating and cooling was found earlier in the Langmuir films of diacetylene derivatives polymerized on water surface and after deposition into a solid substrate [15]. Our case differs from this by using the dye covalently bonded to polymer. We have studied absorption spectra and spectral dependencies of Stark effect in LB-films of polyglutamylcarbocyanines and suggest an explanation of reversible colour changes based on structural transitions of macromolecule.

In order to obtain the spectral dependence of Stark effect we apply to the film an AC voltage and measure the small relative changes in optical transmittance  $\Delta T/T$  using a lock-in amplifier. On the second harmonic of applied field with amplitude  $E$  the optical density  $D$  and amplitude value of  $\Delta T/T$  are linked with the relationship [16] :

$$-\frac{1}{\ln 10} \frac{\Delta T}{T}(\nu) = \frac{\nu E}{2hc} \left[ \frac{(\Delta\mu)^2}{hc} S_2 \frac{d^2}{d\nu^2} \left( \frac{D}{\nu} \right) + \Delta\alpha \frac{d}{d\nu} \left( \frac{D}{\nu} \right) \right] \quad (1)$$

where  $c$  and  $\nu$  are light velocity and wavenumber,  $h$  is Plank's constant,  $S_2$  is expressed through the average parameters of the molecular distribution over the angle  $\vartheta$  between the chromophore axis and film normal as follows:

$$S_2 = \frac{\langle \cos^2 \vartheta \rangle - \langle \cos^4 \vartheta \rangle}{1 - \langle \cos^2 \vartheta \rangle} \quad (2)$$

$\Delta\mu$  and  $\Delta\alpha=[\Delta\alpha_1S_2+\Delta\alpha_1(1-S_2)]$  are the changes in a dipole moment and polarizability upon excitation (indices l and t denote the longitudinal and transverse components of the tensor respectively). Therefore, having  $S_2$  and the curves  $\Delta T/T(\lambda)$  and  $D(\lambda)$  from independent measurements one can calculate the molecular parameters  $\Delta\mu$  and  $\Delta\alpha$ .

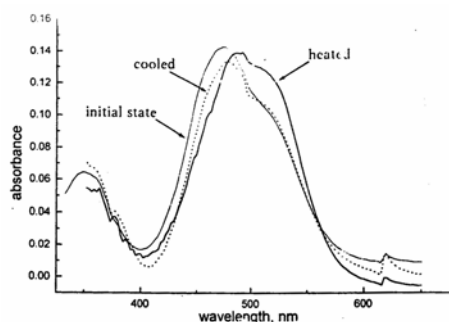


Figure 2. Absorption spectra of the film in initial, after-heated and cooled states.

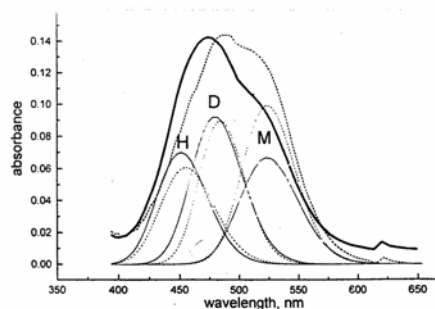


Figure 3. Decomposition of absorption spectra in initial (solid line) and after-heated (dashed line) states into three Gaussian shaped bands, attributed to monomers (M), dimers (D) and H-aggregates (H).

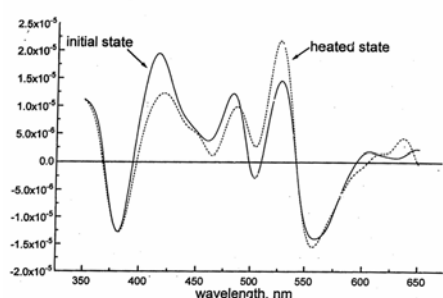


Figure 4. Spectral dependences of Stark effect in initial and after-heated states.

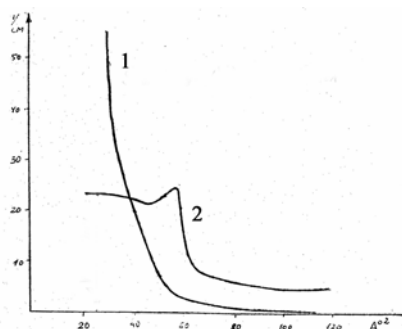


Figure 5. P-A-Isotherm for compound 3 (1). Electropotential curve at 450mV (2).

The Stark spectra analysis is done using a special software. At first, the total absorption band is decomposed into three separate Gaussians (fig. 4). Second, the experimental Stark spectrum is fitted by the sum of linear combinations of the first and second derivatives of each Gaussian to obtain a best agreement. Finally, from the coefficients at the derivatives the values  $\Delta\mu$ , and  $\Delta\alpha$  are calculated for each band (fig.4).

Colour transitions in investigated films with change of temperature can easily be seen by naked eye. The spectra of optical density of investigated films in initial (room temperature), after-heated ( $t=80^{\circ}\text{C}\rightarrow 20^{\circ}\text{C}$ ) and after-cooled states ( $-20^{\circ}\text{C}\rightarrow 20^{\circ}\text{C}$ ) are shown in Figure 2. The observed maximum value of spectral shift is about 40 nm for the temperature change of about  $100^{\circ}\text{C}$ .

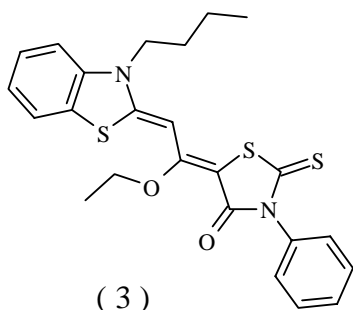
It was shown in [14] that LB film with n-Glu-dye (1) is formed as molecular associates: dimers and H-aggregates (side-by-side interaction), which are highly ordered. Each kind of associates makes a contribution to the absorption spectrum. The spectral changes can be explained from the assumption that on heating the relationship among the concentrations of monomers, dimers and H-aggregates varies due to a breakdown of a part of highly ordered aggregates into monomers. Figure 3 shows the decomposition of the absorption spectrum into three Gaussian shaped bands, attributed to monomers (M), dimers (D) and H-aggregates (H) (whole spectrum and component peaks, corresponding to the after-heated state, are displayed by dashed line). After heating the magnitude of H-aggregates peak decreases while the monomer band grows.

It is known that high level of aggregation is accompanied by an increase of the change of the molecular polarizability  $\alpha$ . The values of  $\Delta\alpha$ , corresponding to the different types of molecular aggregates at room temperature before and after heating have been calculated from the absorption and Stark spectra (the latter is shown in fig. 2) using the equation (1). The parameters  $S_2$  were taken from [14] where they have been found by measurement of out-of-plane dichroism. The results are presented in the TABLE 1.

Table 1. Molecular polarizability upon excitation.

State	Change of molecular polarizability upon excitation, A3.		
	Monomers	Dimers	H-aggregates
Initial	0.63	0.58	1.51
Heated	0.41	0.32	0.52

One can see that the found values of  $\Delta\alpha$  are quite different the heating. In fact we had to find the same values for monomers. The discrepancy of about 20% for monomers is explained by inaccuracy of the value  $S_2$  for the initial state. After heating the distribution function of monomers could be changes in a such way so the value  $S_2$  decreases to be closer to the value of 0.2 (isotropic distribution). The last assumption explains the smaller value of  $\Delta\alpha$  found after heating. For the H-band  $\Delta\alpha$  decreases by factor of 2.9. Such a big factor can only be explained by H-aggregates decomposition.



Electrothermal effect was detected for another type of carbocyanine dyes (3) what was tested as ultrathin coating for electroconductive systems [17]. LB-films from polyglutamyl-merocyanine were examined as colour-change electroconductive films. Merocyanine dye (3) was used and in mix with polyglutamic acid (content of dye 45%) and without polymer. The longitudinal and cross-conductivity were investigated. The longitudinal electroconductivity has appeared in present of donor or acceptor (dicyanine complexes). The cross conductivity existed in present of acceptor after doping of iodine. All processes were accompanied by decolourizing (during 30 sec.) and thermal effect.

Electroconductivity processes are accompanied of heating (about 35°C) and decolourizing of compound (3) after doping  $I_2$  in presence of acceptor. Alone dye does not detect any thermochromism by heating only. Probably, electrothermal stimulation is specific property for this substance in LB-film doping  $I_2$ . UV spectra leaves unchanged after multitude electrotests. System: polyglutamic acid / dye (3), dicyanin acceptor,  $I_2$  - is stable and we can notice changes of colour and thermal effect under electricity current multitudes without any deformation of LB-films and decomposition of dye.

Earlier we showed the synthesis of regular PGA containing different dyes-carbocyanes, coumarines, acridines in fixed position in side chains [18]. Introduction of substitutes depends upon steric factors- size, shape and flexibility of entrance molecules and also reactivity of dyes functional groups. In this part of work introduction of functional amines of Fluorescein and Rhodamine both together and separately in polyglutamic acid with MM ~4200 ( $n \sim 40$ ) was carried out. The contents of dyes were 33% for Flu, 26% for Rhod. When we introduced both Fluorescein and Rhodamine (Figure 6, compounds 4, 5) the general content was ~24 %. By other words, Fluorescein can occupy one aminoacid unit and three units left free. Rhodamine can occupy every fifth unit only because of steric hindrance. When combined introduction of two dyes was carried out their distribution had random character, however, quantative analysis by UV and NMR data corresponded with alternated species.

Quantum chemical methods were applied for optimization of the geometry of possible Structures involving non-covalent bonded Assemblies. Analysis of the fine details of the atomic interaction in terms of the electrostatic potential was realized. The presence of free  $\gamma$ -carboxylic groups in macromolecule and many amide bonds in backbond supposes a great number of hydrogen-bonding centers in PGA itself. Dye molecules have also possibilities for hydrogen bonds formation, especially it relates to Flu which has free hydroxy groups. Both Flu and Rhod can exist in carboxylic or carbonyl form due to easily isomerized carboxylic group [19]. Besides interconversion of these carboxylic groups can lead to both charged and discharged forms of dye molecule. The possibility of ionic form exists also for PGA. Thus it was necessary to take into consideration the presence of many centers of intra- and intermolecular contacts which can implement at the expense of hydrogen bonding, Van-der-Waals and electrostatic interactions. In case of Rhodamine the possibility of hydrophobic interactions due to sterically screened diethylamino groups should be taken into account as well. CD data showed that in all cases substituted PGA can exist in  $\alpha$ -helix conformation at 20°C, pH=6÷7 in hexafluoropropanol, Dimethylsulfoxide, Dimethylformamide, though helix-coil transition at temperature or pH changes was not ruled out.

Obviously, the strand formation from two  $\alpha$ -helixes happens due to van der Waals interaction and H-bond system. When  $\alpha$ -helixes interact their both structures and the torsion angles are changed and one helix entwines the other one, so the strands from two  $\alpha$ -helixes could be formed. We suppose further possible inter-strand interaction to bundle from 2 strands, at least, so they have a lot of contacting centers involving H-bonds. Side-chain interactions would be more preferable than back-bond contacts in this case [20]. Prevailing arrangement including an orientation of side-chain substitutes allows chromophores to organize as associate forms (i.e. dimers). General interaction can result in network

production with different geometry. Most probably, Rhodamine substituted PGA has less features for Assemblies because hydrophobic interaction can partly lead to the disordered structures [21].

It was useful to consider optical properties of luminescent PGAD in Solutions. Fluorescein derivatives detect extremal complicated fluorescent behaviour in water, including extinguishing, so we did not study water Solutions in details. Alcohol Solutions of Rhodamine are used as liquid laser medium, however dynamic decrease of excited State of Fluorescein on protein Substrates in alcohols is known [19]. Present optical measurements were carried out in dimethylsulfoxide Solutions of luminescent PGAD, although identical data were obtained for fresh prepared methylalcohol Solutions as well.

The data of optical Spectra have demonstrated notable features for Flu-containing PGA, fig.3. We compared Absorption spectra of initial dyes, model compounds where alone dye molecule immobilized on PGA, and PGA maximal containing luminophores. Small shift (3 nm) from 521 nm to 523 nm to long wave field was noticed for n-Glu-Flu, containing 0.25% mol of dye and 33% molar % fluorescein, but last compound detected weak absorption 499nm, fig. 3(a). Strong splitting was detected for n-Glu-Flu containing Fluorescein 33 molar % in presence of TEA (10 %): 3 peaks 523nm, 490 nm, 462 nm. We explain these phenomena by different forms of dyes, involving association of side substituents in macromolecules. Absorption with  $\lambda=523$  nm can be related to charged forms of Fluorescein that can be released by base addition (salt formation) (Figure 6).

Illumination of n-Glu-Flu could be detected in presence of triethylamine only with shift from 565 nm to 552 nm to short wave field. Uncharged binding Fluorescein possesses the ability to self-quenching that is described for its complexes with protein substrates [19]. Obviously we have similar conditions for Fluorescein immobilized on polyglutamic acid, where molecular association predominated. Basic triethylamine breaks associative links and charged form of dye illuminates.

Absorption and illumination of n-Glu-Flu-Rhod has analogical properties. In particular in DMSO/TEA absorption peak 523 nm (Flu) is kept. Illumination with  $\lambda=552$  increase, strong illumination with  $\lambda=569$  appears at the same time. Last peak is situated near initial Fluorescein illumination (565 nm). Present picture confirms influence of Substitute nature to properties of all System. Neighbouring Rhodamine has less binding centers and can block partly H-bonding of side groups. However its illumination decreases in basic media that can be invoked by Rhodamine aggregates destruction. All these facts confirm obliquely presence of different Assemblies of PGAD in Solutions. In this connection Aggregation of side chains is more preferable for geometry of macromolecule and intermolecular interaction may be realized to Strand formation that is in accordance with computer models.

CD spectra in DMSO have shown helical conformation for PGAD. Addition of TEA up to 10 % weight invoke insignificant decreasing of curve intensity, fig.4. So helical conformation is presented in contorted state even.

Earlier we have shown high-ordered Aggregation in monolayers for regular rigid-rod PGA containing carbocyanine dyes residues, for example H-aggregates of PGA, with carbocyanines and their reorganization under UV-irradiation by out of plane dichroism method are investigated. In present luminescent PGAD formed monolayers with good Stability on Subphase and by transfer to quartz Substrate. Detailed analysis of P-A isotherms in area of small Surface pressure gives information about the molecule aggregation Simulation of area of less discharge monolayer for n-Glu-Flu when molecule section is less than medium area is represented by Figure 6.

For Langmuir layers of n-Glu-Flu Limit area value per molecule ( $A_{lim}$ ), equal to  $93 \text{ \AA}^2$  were found. The number of molecules in kinetical unit (n) defined from the linear extrapolation of experimental dependence to zero value P. By other word at low pressures n-Glu-Flu Langmuir layer on the water surface consists of molecular aggregates containing on average 29 molecules, effective area ( $A_0$ ) of one molecule in aggregate is  $106 \text{ \AA}^2$ . Similar calculation estimates 2 mol / Aggregate for n-Glu-Rhod and 4mol/ Aggregate for n-Glu-Flu-Rhod. Taking into consideration the fact that error in computation is equal to  $\pm 2\text{mol/Aggr}$  it can be proposed the strand formation and inter-strands interaction as the most probable. So n-Glu-Flu aggregation produces 7 bundles from 2 strands (28 molecules), n-Glu-Rhod forms strand (2 mol.)n-Glu-Flu-Rhod produces bundle from 2 strands (4 mol).

Obviously, the strand formation from two  $\alpha$ -helices happens due to Van-der-Waals interaction and H-bond system. When  $\alpha$ -helices interact their both structures and the torsion angles are changed and one helix entwines the other one, so the strands from two  $\alpha$ -helices could be formed. We suppose further possible inter-strand interaction to bundle from 2 strands, at least, so they have a lot of contacting centers involving H-bonds. Side-chain interactions would be more preferable than back-bond contacts in this case [21]. Prevailing arrangement including an orientation of side-chain substituents allows chromophores to organize as associate forms (i.e. dimers). General interaction can result in network production with different geometry. Most probably, Rhodamine substituted PGA has less features for assemblies because hydrophobic interaction can partly lead to the disordered structures [22].

Bundle formation motif is in accordance with proposed Self-Organization in Solution and doesn't contradict proposed models of modified polyglutamic acid packing.

FTIR spectra of the monolayers for n-Glu-Flu and n-Glu-Rhod in the C=O stretching regions were developed as well. The amide I and amide II are represented as two bands at  $1662 \text{ cm}^{-1}$  and  $1550 \text{ cm}^{-1}$  which relate to  $\alpha$ -helical structures. The stretching band of COOH side chains of PGA is observed at  $1730 \text{ cm}^{-1}$ . Taking into account rigid helical rods of initial substances, it was suggested helical packing in monolayers. Combining experimental data for integrated intensities ratio of amide I and amide II in FTIR for our samples, number of molecules in Aggregates in monolayers and analogy with regular structure PGAD from [14] we can propose high ordered aggregation of polyglutamic acid containing luminescent markers. Absorption spectra of LB-films (40 monolayers) have  $\lambda_{max}=523\text{nm}$  for n-Glu-Flu and  $\lambda_{max}=566\text{nm}$  for n-Glu-

Rhod, that corresponds to  $\lambda_{\max}$  of binding dyes. Preliminary we have obtained results demonstrated influence of pH media on the luminescence of multilayer film (n=40) n-Glu-Flu. Illumination was noticed when film was replaced to basic atmosphere with pH > 10. Fluorescein contained polyglutamic acid LB-films do not illuminate on neutral media. This sensor feature for ultra thin of modified polyaminoacid is object of our futural research.

### Photoswitches on the Polypeptide Basis

Cation-binding ability of crown-compounds by photoisomerization of Spiropyranes [23] is tested for photo-switchable elements construction.

Hydroxyspiropyranes - mono- and bis-functional (comp. 6, 7) were introduced to cyclic polypeptide, containing diaminodibenzo-18crown-6. Previously 18-member cyclic n-Glu was connected with crown-18 and adipinic acid as spacer. Monohydroxyspiropyranes (6) produces one<sup>^</sup>cpx with cyclic compounds. (ratio 1:1). Bis-hydroxybridged-double spiropyrane (7) was introduced in cyclopolypeptide with 18-crown-6 in ratio 1:1, two complexes were isolated at least. Spectral and kinetic characteristics of complexes were considered in Solutions in compare with initial photochrome. Non-polar solvents assist to stable complexes. Absorption Spectra has weak shift 580 nm after irradiation, un-colorness reaction is not in accordance with first order. Solvatochromizm influences on behavior of all complexes. The increase of thermal relaxation for coloured forms depends on the microequipment and polarity of used solvents. Possible architecture (inter- and intramolecular) of obtained complexes are suggested on the basic of DSC and X-ray data.

Spiropyran can coordinate with crown-metal-ions in “open form” and let on the metallo-ion in closed state, f. e. irradiation stimulate reversible “switch-process”. Further few attempts to construct “Shuttle Systems” with optical switching were existed. Two types of “Stick co-polymers” were applied and tested with different stop-points. The principal possibility to fabricate photodriving Rotaxane is demonstrated.

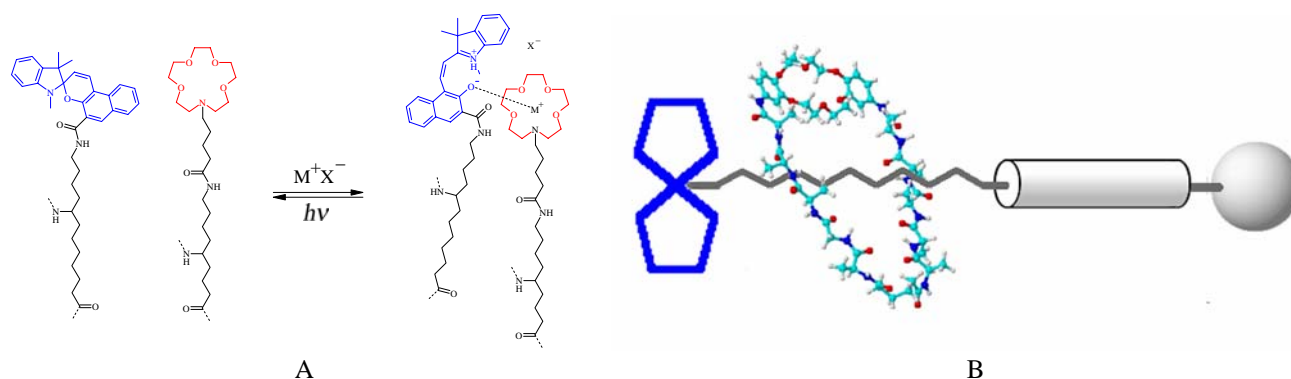


Figure 9. A - cation-binding by photoisomerization; B - model of photocontrolled shuttle.

NMR spectrum of product after visible-light irradiation confirms crown-Me (Li) ion complexation, with open phenolate-anion of DSp, that correlates with AES data as well.

Interlocked structures are convient models for molecular machines fabrication with external control [8]. Small size of cyclopeptide (not more than 8 aminoacid units) permits to construct “Shuttle” model with DSp as Stop-point. Photoswitchable process is considered for interlock-Systems: alkyl or alkyl/peptide chain is situated inside cyclopeptide/ crown with DSp on the end. Using of cycloglutamic acid as spread has advantage, so this construction can exist in monolayer and produce aggregate (5 mol/aggr). Photochromizm study on LB-films is continued up to now, Figures 8, 9, 10.

### Oligoaminoacids with Cyclophosphazene Core (Star-Like Design)

In this study, we focused on the well-ordered and highly-oriented structures constructed on a cyclotriphosphazene core. Hexakis(4-aminophenoxy)cyclo-triphosphazene has been used as initiator for the ring-opening polymerization of *N*-carboxyanhydride of  $\gamma$ -methyl and  $\gamma$ -benzyl L-glutamates. The structure and conformational properties of the resulting oligopeptide chains were investigated by FT-IR,  $^1\text{H}$  NMR, and CD spectroscopies.

Pyrene moieties were attached to the end of peptide chains as fluorescent chemosensors (Figure ).

The aiming molecules have been prepared by covalently anchor peptide chains to the cyclotriphosphazene by using both peptide-coupling techniques and/or ring-opening polymerization of *N*-carboxy anhydrides of L-glutamates. The length of the peptide chain was well controlled by initial *N*-carboxy anhydride – initiator molar ratios (at average – 8, 20 or 40 for each ray).  $^1\text{H}$  NMR spectra of the start hexakis(4-aminophenoxy)cyclotriphosphazene showed specific chemical shift of aromatic protons of non-substituted aminophenoxy groups at 6.4-6.5 ppm, which disappeared in the result molecule. This indicates that all amino groups initiated ring-opening polymerization of *N*-carboxy anhydrides without steric hindrance[24].

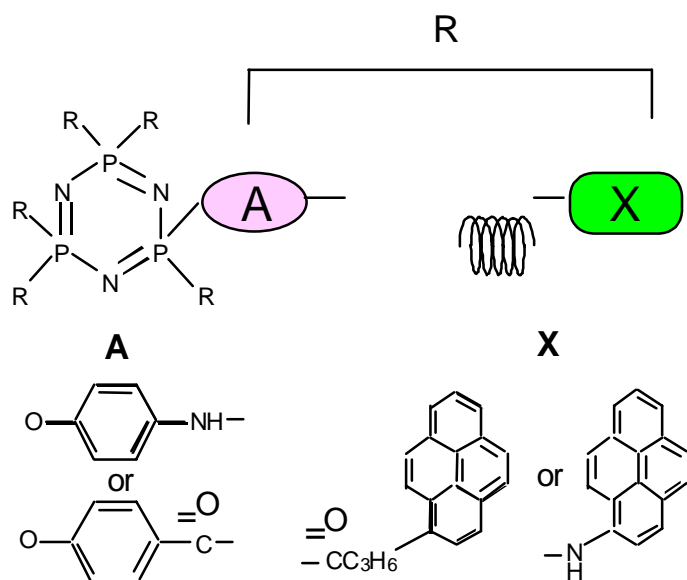


Fig. 11 Structure of six-helix bundle molecule

ferent nature are used. These light-sensitive systems possess liotropic liquid crystalline properties that open new perspectives for special sensors constructions. Recently it was reported new photoactive cyclotriphosphazene / peptide systems [25] where azo-group was introduced to peptide chain. This compound produces organo-gel by immobilization on SiO<sub>2</sub>. Photosensitive trigger detects switch possibility that permits to control optical properties of multi-functional adaptive inorganic-organic hybrids.

## Experimental

### Materials

Polyglutamic acid (MM 4200 and 6000) was synthesized by N-carboxyanhydride method (NCA) in our laboratory from N-benzyloxycarbonyl- $\gamma$ -methyl ester of L- $\alpha$ -glutamic acid, purchased from "Sigma" in accordance with [26] with following hydrolysis of ester groups [27], molecular mass was defined by exclusion chromatography. Dyes Flu and Rhod were bought from "Fluka" and then were converted into functional amines by organic chemistry methods, carbocyanines were synthesized in our laboratory [18]. Modification of polyglutamic acid in side chains by dyes was carried out by dicyclocarbodiimide method [18]. The degree of % mol bonding was estimated by NMR and UV methods.

### Measurements and equipment.

Optical measurements were held using Hitachi EPS UV-SP spectrometer in UV range. CD spectra were recorded on a Jasco J 500A spectropolarimeter in DMSO and HFP. They are expressed in terms of molar ellipticity  $[\theta]$ , based on the means residue molecular weight. Intrinsic viscosities  $[\eta]$  were determined with Desreux- Bishoff dilution viscometer at 25C in DMFA. Fluorescent spectra were recorded using the multichannel optical analyzer OMA-2 with nitrogen laser source (337nm) and Edinburgh Instruments spectrofluorimeter 199 by the time-correlated single photon counting technique. The FT-IR spectra were performed on a Bruker IFS 113 V spectrometer with a mercury-, cadmium-tellurium (MCT) detector.

The studying of Langmuir layers of polyglutamic acid bearing with dyes was carried out by the method of measuring dependences of surface pressure on area per one molecule under compression of monolayer (P-A compression isotherms).

Y-type Langmuir-Blodgett films for compounds 1, 2, 3, 4 consisted of 15 monolayers prepared using Langmuir through of local design by subsequent transfer of monolayers from a surface of tridistilled water onto fuser quartz with SnO<sub>2</sub> transparent electrode at room temperature and surface pressure of 15 mNm<sup>-1</sup>. A counter semitransparent electrode has been made by evaporation of aluminum in a vacuum. Absorption and Stark effect spectra of the samples were obtained with spectrophotometric set-up of local design.

Merocyanine dye incorporated to polyglutamic acid 45% content, fabricates stable multilayers by Langmuir-Sheffer method; surface pressure is  $\pi=20$  mNm/cm,  $S_{\text{mol}}=40\text{\AA}^2/\text{mol}$ .

The electropotential leap is 450mV. Bright colour is noticed for multilayers from 1 to 200 monolayers. A longitudinal electroconductivity is studied for samples applied to a comb getting chromeous dust. A cross-linked conductivity is studied for quartz and polymers plates with SnO<sub>2</sub>/Al electrode pair. The doping of free I<sub>2</sub> is used.

LB-films contained 200 monolayers have been transferred on solid polymer plate with good optical grades. Reversible decolourizing and thermal effect were noticed by electroconductivity ( $I \sim 0.3\text{A}$ ), using SnO<sub>2</sub> and Al-electrode pair. Separate weak heating ( $\sim 45^\circ\text{C}$ ) invoked colour-change after 20 min. only. Electrothermal simulation is supposed to change colour of initial LB-films of polyglutamylmerocyanine in short time.

Universal Voltmeter B7-32, 2, form XB2.710.027FO (Russia),  $U=10^{-4} - 10^{-3}\text{V}$ . B5-43A, B5-44A, B5-45A, form 3. 233. 001FO.

### Computer modeling.

The molecular weight estimated by size exclusion chromatography was close to the calculated value and the molecular weight distribution was reasonably narrow ( $M_w/M_n < 1.3$ ). CD spectra of all compounds both in certain solvents and in casting films showed a maximum at 190 nm and minimums at 208 and 220 nm which are characteristic of right handed  $\alpha$ -helix. Also FT-IR spectra data (absorption at 1652 cm<sup>-1</sup> – amide I, 1548 cm<sup>-1</sup> – amide II) showed that only  $\alpha$ -helices were formed during polymerization of *N*-carboxy anhydride of  $\gamma$ -benzyl-L-glutamate. In the case of  $\gamma$ -methyl ester, however, a slight amount of  $\beta$ -sheet structure (absorption at 1630 cm<sup>-1</sup> – amide I, 1525 cm<sup>-1</sup> – amide II) was observed in addition to the  $\alpha$ -helix absorption. These observations were as well supported by <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra.

Principally introduction of luminophores is possible from 1 mol to 6 mol, being fragments of dif-



Quantum chemical non-empirical Hartree-Fock calculations of functional fluorescein, rhodamine and oligo-L-glutamic acid (n=1-4) were performed using 6-31G\* and 6-31G\*\* basis sets. Computer program GAMESS has been used. Electrostatic potential, parameters of Mulliken population analysis (bond orders, atomic charges) were calculated. Geometrical parameters of n-poly-L- $\alpha$ -glutamic acid modified with dyes were then optimized by the force field method (MM2) in a dipole approximation as well as by the MNDO method. Parameters of molecules derived by non-empirical Hartree-Fock calculation were taken as initial guess for optimization.

Found geometry parameters of  $\alpha$ -glutamic acid oligomers (n=1-5) were used for construction of the  $\alpha$ -helix and  $\beta$ -sheets. General view on some molecular fragments derived is presented in Supporting Information. Chains with n since 10 till 40 were considered. It was revealed that the flat  $\beta$ -sheet is already distorted at n=10. The calculations showed that modification of n-Glu by Rhod or Flu also lead to distortion of n-Glu  $\alpha$ -helix.

Electrostatic potential in functional fluorescein and rhodamine showed the deep negative areas surround the regions of lone electron pairs of nitrogen and oxygen atoms. These minima can be associated with sites of the maximal probability of electrophilic attack, which leads to the amide bond formations with polyglutamic acid.

According to [17], n-poly-L- $\alpha$ -glutamic acid modified with dyes can be presented as rigid-rod or cylinder with inner diameter neglecting side chains about of 6 Å. Outer diameter with attached luminophores is approximately of 12.7 Å, while helical rod is 103 Å for n=40. Each turn of the helix may involve 3.6 residue.

## Conclusion

The various polyaminoacid derivatives are enable to construct different molecular devices with specific properties and molecular architectures: assemblies of rigid-rods; rotaxanes; template-supported structures. They can function in solution, film, gel, liquid crystals. Introduction of light-sensitive fragments leads to nanoscale stimuli responsive systems assisted by conformational rearrangements that is existed in natural proteins. Adaptive multifunctional systems on the polyaminoacid basis have future perspectives for biomimetic intelligent materials creation.

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## References

1. Lehn, J.-M. Where It Is and Where It Is Going, Supramolecular Science, Ungaro R., Dalcanele E., Eds., Kl. Acad. Publ. 1999, 287.
2. Hoffman, A. Macromol. Symp. 1995, 98, 645-664.
3. Wegner, G.; Remmers, M., Int. Ullman's Encyclopedia of Industr. Chem, 5 ed., VCH Weinheim, 1995, A26, 2.6.1., 731-735.
4. Menzel H., Polymer Mat. Encyclopedia, ed. J. Salamone, CRS Press, N.J. 1997.
5. Pieroni, O.; Fissi, A.; Popova, G.; Prog. Polym. Sci., 1998, 23, 81-123.
6. Lane A. S., Leigh D. A., Murphy A. J. Am. Chem. Soc., 1997, 119, 11092-11093.
7. Meillon J.-Ch., Voyer N., Biron E., Sanschargin F., Stoddart J. F., Angew. Chem. Int. Ed., 2000, 39, 143-145.
8. Balzani V., Credi A., Raimo F. M., Stoddart J. F., Angew. Chem., 2000, 39, 3348-3391.
9. M. Mutter, K. H. Altmann, G. G. Tuchscherer, and S. Vuilleumier, Tetrahedron, 44, 771 (1988).
10. R. M. Ghadiri, C. Soares, and C. Choi, J. Am. Chem. Soc., 114, 825 (1992).
11. T. Sasaki and E. J. Kaiser, Biopolymers, 29, 79, (1990).
12. P. E. Dawson and S. B. Kent, J. Am. Chem. Soc., 115, 7263 (1993).
13. Loup C., Zanta M. A., Caminade A. M., Majoral J. P., Meunier B. Chem. Eur. J., 1999, 5, 3644-3650.
14. S.P. Palto, A.V. Sorokin, S.G. Yudin, G.V. Popova and others. Mol. Mat., 5, 231-235 (1995).
15. H. Tamura, N. Mino, K. Ogawa. Thin. Solid Films, 179, 33-39 (1989).
16. L.M. Bunov, M.I. Barnik, T. Wegrauch, S.P. Palto, A.A. Tevesov, W. Haase. Chem. Phys. Lett., 231, 246 (1994).
17. A. Korigodsky, O. Fedorovsky, N. Matveeva, G. Popova, V. Kireev. Abstracts of III Inter. Symp., "Polymers for Advanced Materials" PAT-95, 11-15. 06. 1995, p. 351, Pisa, Italy.
18. Khalid, M.; Eliseeva, E.; Popova, G.; Yudin, S. Polym. Sci., 1994, 13, 36, 3, 425-429.
19. Krichevski, G. E. Photochemical interconversion of Dyes and light stabilization on colour materials, "Chemistry" Moscow, 1986, Chapter 2.
20. Schmidt, M.W.; Baldridge, K. K.; Boatz, J. A.; Elbert, S.T.; Gordon, M. S.; Jen-sen, J. H.; Koseki, S.; Matsunaga, N.; Nguyen, K. A.; Su, S. J.; Windus, T. L.; Dupuis, M.; Montgomery, J. A., J. Comput. Chem., 1993, 14, 1347-1363.
21. Jones, G.; Villev, V.; Braswell, E.H.; Dan Zhu, J. Am. Chem. Soc., 2000, 122, 388-389.
22. Kashiwada, A.; Hiroaki, H.; Kohda, D.; Nango, M.; Tanaka, T. J. Am. Chem. Soc., 2000, 122, 212-215.
23. K. Kimura, H. Tokuhisa, M. Kuinshige et al. Mol. Cryst. Liq. Cryst., 1994, 246, 173-176.
24. Alekperov D., Sakurai T., Shirosaki T., Popova G., Kireev V., Ihara H. Polymer J., 2003 (in press)

25. Shirosaki T., Ihara H., Alekperov D., Popova G.. ISBOC6 International Symposium on Bioorganic Chemistry, Toronto, Canada, 2002, p. 27.
26. Block, H. Poly( $\gamma$ -benzyl-L-glutamate) and Other Glutamic Acid Containing Polymers', Gordon and Breach: New York, 1993.
27. Grinshteyn, J.; Vinitz, M., Chemistry of aminoacids and peptides, M., 1965, 703-711.